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MOLECULAR MECHANISMS UNDERLYING THE PLASTICITY OF MUSCLE CONTRACTILE PROPERTIES WITH TEMPERATURE ACCLIMATION IN THE MARINE FISH *MYOXOCEPHALUS SCORPIUS*

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Summary

Single fibres were isolated from the fast myotomal muscle of the short-horned sculpin (*Myoxocephalus scorpius* L.). Fish were acclimated to either 5 °C (10 h:14 h light:dark) or 15 °C (12 h:12 h light:dark) for 1–2 months. Isometric tension (F_{max}) and unloaded contraction velocity (V_{slack}) were determined in maximally activated skinned fibres over the range 0 to 20 °C. Fibres isolated from 5 °C-acclimated and 15 °C-acclimated fish failed to relax completely following activations at 15 °C and 20 °C respectively. In 5 °C-acclimated fish, F_{max} increased from 75 kN m^{-2} at 0 °C to 123 kN m^{-2} at 10 °C and was not significantly higher at 15 or 20 °C. The relationship between F_{max} and temperature was not significantly different for cold- and warm-acclimated fish. V_{slack} was around 2.8 fibre lengths s^{-1} in both acclimation groups at 0 °C, but it increased at a significantly faster rate with temperature in 15 °C- than in 5 °C-acclimated fish. At 20 °C, V_{max} was significantly higher in 15 °C-acclimated (8.7 fibre lengths s^{-1}) than in 5 °C-acclimated fish (5.3 fibre lengths s^{-1}). In order to investigate the molecular mechanism(s) underlying changes in V_{max}, myosin was purified by ion-exchange chromatography. No difference in myosin heavy chain composition could be detected on the basis of peptide mapping with four different proteolytic enzymes. Two-dimensional polyacrylamide gel electrophoresis revealed no myofibrillar protein isoforms unique to either acclimation temperature. However, the ratio of myosin alkali light chain contents ($LC3_f$:$LC1_f$), as determined by capillary electrophoresis, was significantly lower in muscle from 15 °C-acclimated (0.73) than from 5 °C-acclimated fish (1.66). The results suggest that changes in V_{max} are achieved *via* altered expression of myosin light chains independently of myosin heavy chain composition. In support of this hypothesis, the myofibrillar ATPase activity of fast muscle was not altered by temperature acclimation.

Key words: teleost, skinned muscle fibres, myosin light chains, temperature acclimation, *Myoxocephalus scorpius.*

Introduction

Fry and Hart (1948) measured the maximum swimming speed that goldfish (*Carassius auratus* L.) could maintain in a rotating chamber over 2 min and found that it was a function of both the environmental temperature and the thermal history of the fish. Swimming of such short duration, at relatively high speeds, is likely to involve the recruitment of both red and white muscle fibres. For 5 °C-acclimated fish, the maximum cruising speed increased to an optimum at 18 °C and then declined. In contrast, fish acclimated to 35 °C could not swim below 20 °C and showed a performance optimum at 38 °C, indicating a trade-off between high- and low-temperature performance with acclimation. In a recent study of the escape response in goldfish, the kinematics and thermal optimum of C-starts were similarly found to be modified by 4 weeks of temperature acclimation (Johnson and Bennett, 1995).

Temperature acclimation results in a major change in muscle phenotype in goldfish and other carp species (Johnston *et al.* 1990). Several weeks of cold-acclimation is associated with an increase in the relative volume of red muscle fibres (Johnston and Lucking, 1978; Sidell, 1980). Mitochondrial volume density is also inversely related to acclimation temperature (Johnston and Maitland, 1980). The increase in mitochondrial volume density with cold acclimation is thought partially to offset the effects of low temperature on rates of oxidative phosphorylation (Johnston and Maitland, 1980) and the diffusion of metabolites (Sidell and Hazel, 1987). Following cold-acclimation, red muscle fibres express myosin isoforms characteristic of faster-contracting fibre types, leading to a small increase in maximum contraction velocity (V_{max}) at low temperatures (Langfeld *et al.* 1991).

Myofibrillar ATPase activity in the white muscle fibres of goldfish, carp and several other cyprinid fishes is several-fold

higher at low temperatures in cold- than in warm-acclimated individuals (Johnston *et al.* 1975; Sidell, 1980). Studies with isolated muscle fibres have shown that both unloaded contraction velocity (V_{max}) and the maximum isometric stress (F_{max}) are almost twofold higher at low temperatures in cold- than in warm-acclimated carp (Johnston *et al.* 1985, 1990). However, following cold-acclimation, skinned fibres failed to relax completely following maximal activation at high temperatures, again suggesting a trade-off between low- and high-temperature performance. The molecular basis of these changes in muscle contractile properties in the carp includes altered expression patterns of myosin light chain (Crockford and Johnston, 1990) and myosin heavy chain genes (Gerlach *et al.* 1990; Hwang *et al.* 1991). Cold-acclimation in carp also resulted in faster relaxation rates of isolated muscle fibres at low temperatures and an increase in sarcoplasmic reticulum Ca^{2+}-ATPase activity relative to that of warm-acclimated fish (Fleming *et al.* 1990).

Some plasticity of swimming performance and muscle phenotype with seasonal temperature change may be relatively common in eurythermal fish (Lemmons and Crawshaw, 1985; Sisson and Sidell, 1987; Egginton and Sidell, 1989). Beddow *et al.* (1995) studied predation fast-starts in the short-horned sculpin (*Myoxocephalus scorpius*) and found that at 15 °C the maximum velocity was 33 % higher in 15 °C- than in 5 °C-acclimated fish. This was sufficient to increase the percentage of successful attacks from 23 to 72 %. Fast-start performance also improved at low temperatures following cold-acclimation (I. A. Johnston and C. E. Franklin, unpublished results). Johnston *et al.* (1995) measured the power output of isolated fast muscle fibres with the work loop technique using the strain fluctuations and stimulation patterns observed during the fast-start. At 15 °C, the average power output during the first tail-beat was 6.3 W kg^{-1} wet muscle mass in 5 °C-acclimated fish and 23.8 W kg^{-1} wet muscle mass in 15 °C-acclimated fish. Although live muscle fibres from 5 °C-acclimated fish had lower maximum isometric stresses (F_{max}) at 15 °C than fibres from 15 °C-acclimated fish, force was increased twofold on depolarisation with a high-K^+ solution (Beddow and Johnston, 1995). These results suggest that there was some failure of activation and/or excitation–contraction coupling at high temperatures in the muscle of cold-acclimated fish. In contrast to carp, temperature acclimation did not modify muscle relaxation rates in the short-horned sculpin (Beddow and Johnston, 1995).

In the present study, on the short-horned sculpin, the use of skinned fibres has enabled us to investigate the effects of thermal acclimation on the contractile properties of the myofibrillar proteins directly and independently of the effects of temperature on motor endplates and muscle membranes. The temperature-dependence of V_{max}, but not F_{max}, was found to vary with the thermal history of the fish. In order to investigate the molecular mechanism(s) underlying changes in V_{max} with temperature acclimation, we characterised myosin heavy and light chain subunits. The results indicate major differences in both the contractile responses and the underlying molecular mechanisms of temperature acclimation between the short-horned sculpin and cyprinid species.

Materials and methods

Fish

Short-horned sculpin (*Myoxocephalus scorpius* L.) were caught locally throughout the year by trawling or by trapping in lobster pots. Fish were maintained in filtered seawater aquaria at ambient temperature (3–15 °C) for 7 days with a 12 h:12 h light:dark photoperiod. They were subsequently acclimated to either 5 °C (10 h:14 h light:dark) or 15 °C (12 h:12 h light:dark) for 1–2 months. The fish were matched for size in order to avoid any potential confounding scaling effects (Altringham and Johnston, 1990). The standard lengths and body masses of the fish used were 19.3±2.2 cm and 180.8±42.2 g respectively (mean ± S.D., N=23) for the 5 °C-acclimated group and 18.4±1.7 cm and 164.4±42.5 g respectively (mean ± S.D., N=24) for the 15 °C-acclimated group. They were fed *ad libitum* on a diet of diced squid and fish, supplemented with live shrimp (*Crangon crangon*). Fish were killed by a blow to the head followed by pithing and transection of the spinal cord.

Isolation of muscle fibres

Strips of fast muscle were dissected from the dorsal region of myotomes 3–6, counting from the head. Single muscle fibres were isolated from the strips under silicone oil (MS 550, BDH, Poole, England) on a cooled dissection stage (0–4 °C) as previously described (Altringham and Johnston, 1982). Fibres were mounted between two stainless-steel hooks and secured using acrylic–acetone glue (Altringham and Johnston, 1982). One end of the preparation was attached to a force transducer (AME 801, Horton, Norway; sensitivity 0.5 mN V^{-1}) and the other to an electric motor (MFE model R4-077, Emerson Electronics Bourne End, Bucks, UK) and a custom-built servo-system. The apparatus consisted of three water-jacketed chambers (±0.2 °C) mounted on a two-way micromanipulator which enabled the preparation to be rapidly transferred between solutions and sarcomere length to be adjusted. The fibres were skinned for 15 min by immersion in relaxing solution containing 1 % (w/v) Brij 58 (polyoxyethylene 20 cetyl ether). The composition of the relaxing solution was (in mmol l^{-1}): Pipes, 25; EGTA, 15; $MgCl_2$, 7.4; ATP, 6; phosphocreatine (PCr), 10; 25–50 i.u. ml^{-1} creatine phosphokinase (1 unit will transfer 1.0 μmol of phosphate from phosphocreatine to ADP per minute at pH 7.4 at 30 °C); pH was adjusted to 7.2 with KOH at 20 °C. Following skinning, the fibre was transferred to relaxing solution for 5 min and the sarcomere length was measured by laser diffraction and adjusted to 2.3 μm. Muscle fibre length and diameter were measured using a microscope with an eye-piece graticule (×200). Fibres were activated in a solution containing (in mmol l^{-1}): Pipes, 40; EGTA, 15; $MgCl_2$ 7.0; ATP, 6; PCr, 15; $CaCl_2$, 15 and 25–50 units ml^{-1} creatine phosphokinase; pH was adjusted to 7.2 with KOH at 20 °C, but was allowed to

change freely with temperature during the experiment. The main ionic species were at the following concentrations: pMg 2.96, pMgATP 2.28, pCa 4.66, pH 7.37, and ionic strength 0.18 mol l^{-1} (values quoted for 0 °C).

The concentration of free calcium was sufficient to activate the fibres fully at each temperature, enabling maximum isometric tension (F_{max}) to be measured. Unloaded contraction velocity (V_{slack}) was determined in fully activated fibres using the slack-test method (Edman, 1979). Once a steady force record had been attained, a series of 1 ms releases was given of sufficient magnitude to abolish tension. Following each release, the time required to take up the slack was recorded on an oscilloscope and the fibre was then re-extended to its resting length. Records were analysed by drawing a line parallel to, and above, the zero force line by an amount equivalent to the noise level of the transducer (15 mV). The time for force redevelopment was measured from the point where the length trace crossed this line. V_{slack} was determined from the slope of a plot of the applied length change *versus* the time taken to redevelop force fitted by a least-squares regression (Johnston and Gleeson, 1987). Since the force declined with successive activations, preparations were only activated once and new fibres from each fish were measured at the different temperatures (0, 5, 10, 15 and 20 °C).

Myofibrillar ATPase activity

Myofibrils were isolated from dorsal fast muscle in a solution containing (mmol l^{-1}): imidazole, 20; KCl, 100; EDTA, 1 (pH 7.2 at 0 °C). The muscle was homogenised using a Polytron blender (three times for 20 s) and centrifuged at 3000 ***g*** for 5 min (4 °C). Two further washes of the pellet were performed in a solution containing (in mmol l^{-1}): imidazole, 20; KCl, 100 (pH 7.2 at 0 °C). The pellet was resuspended in 20 vols of the same solution. Myofibrils were collected by low-speed centrifugation (400 ***g*** for 2 min) as previously described (Johnston and Walesby, 1977). The protein concentration of the myofibrils was determined using the microbiuret method (Itzhaki and Gill, 1964) and adjusted to 3 mg ml^{-1}. ATPase activity was measured at 5 and 15 °C in an assay medium containing (in mmol l^{-1}): KCl, 50; imidazole, 40; $MgCl_2$, 7; ATP, 5, (pH 7.4 at 0 °C) and either $CaCl_2$, 5 (Mg^{2+}/Ca^{2+}-ATPase activity) or EGTA, 5 (Mg^{2+}/EGTA-ATPase activity). The ATPase reaction was stopped by the addition of an equal volume of 10 % (w/v) trichloroacetic acid, and precipitated protein was removed by centrifugation. Inorganic phosphate was measured in the supernatant using the method of Rockstein and Herron (1955).

Analysis of the myofibrillar proteins

Myofibrillar preparation

Muscles samples (3–5 g) were homogenised using a Polytron blender (twice for 10 s) in 20 vols of a solution containing (in mmol l^{-1}) Tris–HCl, 10; NaCl, 100; EDTA, 5; Triton X-100, 0.5 % (v/v). All procedures were carried out at 0–4 °C. Myofibrils were prepared from the homogenate as previously described (Crockford and Johnston, 1995). The final myofibril pellet was washed six times in the isolation solution omitting the Triton X-100 and EDTA.

Myosin purification

Myosin was prepared from the myofibril pellet by extraction for 10 min in a solution containing (in mmol l^{-1}): ATP, 7.5; KCl, 675; $MgCl_2$, 7.5; dithiothreitol (DTT), 1; pH adjusted to 6.4 at 4 °C. The extraction solution was centrifuged at 8500 ***g*** for 20 min. The supernatant was mixed with 12 vols of ice-cold milli-Q water and the myosin precipitated overnight. The crude myosin was collected by centrifugation at 12 000 ***g***, and 200 mg of the pellet was dissolved in 4 ml of sodium pyrophosphate (50 mmol l^{-1}, pH 8.0) with DTT (0.75 mmol l^{-1}) and kept on ice. The myosin was further purified by ion-exchange chromatography (Econo system, Bio-Rad Laboratories, Hemel Hempstead, UK). The sample was loaded onto a Sephadex-Q column (Pharmacia LKB, Uppsala, Sweden) (bed volume 15 ml) equilibrated with sodium pyrophosphate (50 mmol l^{-1}) and DTT (0.75 mmol l^{-1}). The myosin was eluted using a linear gradient of NaCl (0 mmol l^{-1} to 750 mmol l^{-1}). The eluant from the column was monitored at 280 nm and 1.5 ml fractions were collected.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE)

Protein samples (10 mg ml^{-1}) were prepared for SDS–PAGE by heating to 80 °C for 5 min in a solution containing (in mmol l^{-1}): Tris–HCl, 62; SDS, 2 % (w/v); glycerol, 10 % (v/v); 2-mercaptoethanol, 5 % (v/v) and Bromophenol Blue (BPB), 0.001 % (w/v).

One-dimensional electrophoresis

One-dimensional SDS–PAGE was carried out according to the method of Laemmli (1970) using a mini-gel slab apparatus (Bio-Rad Laboratories, UK). The acrylamide concentrations of the resolving gels were 8 %, 12 %, 14 % or 15 %. *N*,*N*′-methylene-bis-acrylamide (Bis) was used as a crosslinker at a concentration of 3.3 %. The stacking gels were made up with either 4 or 5 % acrylamide with Bis as the crosslinker. The apparent molecular mass of the myofibrillar proteins was determined in 12 % SDS–PAGE gels using standard proteins of known molecular mass (Sigma Chemicals, Poole, Dorset, UK; MW SDS 200; range 30–200 kDa).

Two-dimensional electrophoresis

Electrophoresis in the first dimension was by isoelectric focusing (IEF) according to the method of O'Farrell (1975) using mini-PROTEAN two-dimensional cell capillary tube gels (Bio-Rad Laboratories, UK) containing 12 % acrylamide (15 % *N*,*N*′-diallytartardiamide as a crosslinker), 0.1 % Nonidet P-40 (NP40), 8 mol l^{-1} urea and 1 % Pharmalyte ampholytes (pH range 6.5–9.0 and pH range 3–10 in the ratio 1:1 or pH range 9.0–11.0 and pH range 3–10 in the ratio 3:1). Samples were run for up to 2000 V h. Electrophoresis in the second dimension was performed using SDS–PAGE with 15 %

acrylamide resolving and 5% acrylamide stacking gels according to the method of Laemmli (1970). Samples for isoelectric focusing (IEF) were prepared by heating at 30 °C for 60 min in a solution containing: urea, 8 mol l^{-1}; DTT, 10 mmol l^{-1}; NP40, 2% (v/v); glycerol, 10% (v/v); Pharmalyte ampholytes pH range 3–10, 1%; and BPB, 0.001% (w/v). The alkali myosin light chains (LC1 and LC3) and regulatory myosin light chain (LC2) were identified by their pI values and relative molecular masses.

Peptide mapping

Myosin heavy chains were purified using one-dimensional SDS–PAGE on 8% acrylamide gels. The myosin heavy chain bands were cut out after rapid staining in Coomassie Blue G-250 (Crockford and Johnston, 1995) and were mounted onto a 15% SDS–PAGE gel. A modification of the procedure of Cleveland *et al.* (1977) was employed for proteolytic digestion. Samples were overlaid with sample buffer (5 ml) and the protease was loaded onto the gel (2–5 i.u. of protease per sample). Four proteases were used, *Staphylococcus aureus* V8, 1-S chymotrypsin, papain and Lys-C (Sigma Chemicals, Poole). Digestion of the sample was achieved by running the sample through the stacking gel at a low voltage (30 mV) for 50–60 min and then increasing the voltage (150 mV) once the dye front had reached the resolving gel. The samples were run for a further 20–25 min to separate the digestion products.

Staining techniques

Gels were stained for a minimum of 2 h in 0.1% Coomassie Blue G-250, 15% methanol (v/v), 15% ammonium sulphate (w/v) and 3% phosphoric acid (v/v) and destained with 50% methanol. Gels stained with Coomassie Blue were scanned at 600 nm using a Shimadzu CS-9000 densitometer. Silver staining was performed using a commercially available kit (Sigma Chemicals, Poole, Dorset, UK).

Capillary electrophoresis

Samples for capillary electrophoresis were prepared from both fresh and frozen muscle using a modification of the method described by Crockford and Johnston (1995). Samples were homogenised using a Polytron blender and initially washed in 20 vols of (mmol l^{-1}): Tris, 10 (pH 7.0 at 20 °C); NaCl, 100; Triton X-100, 0.5% (v/v) and centrifuged at 1500 ***g*** for 5 min. Myosin was extracted from the pellet for 7 min at 0 °C in a solution containing (in mmol l^{-1}): KCl, 675; ATP, 7.5; $MgCl_2$, 7.5; DTT, 1 (pH 6.4 at 0 °C). The extraction step was stopped by addition of 4 vols of ice-cold Milli-Q water and centrifuged for 10 min at 5000 ***g***. The supernatant was decanted and the myosin allowed to precipitate in 2.5 vols of Milli-Q water for 1 h at 0 °C. The myosin was collected by centrifugation at 5000 ***g*** for 10 min. The myosin light chains were extracted by denaturing the myosin heavy chains in a solution containing (in mmol l^{-1}): KCl, 300; Tris–HCl (pH 8.0), 25; urea, 4000; EDTA, 5; DTT, 2.5, for 30 min at 20 °C. The myosin heavy chains were precipitated by the addition of 10 vols of Milli-Q water and collected by centrifugation at 25 000 ***g*** for 20 min. In order to isolate the regulatory light chain (LC2), some samples were also extracted in a solution containing (in mmol l^{-1}): NaCl, 450; 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), 10; EDTA, 5; Tris–HCl, 20 (pH 8.5 at 4 °C) (Wagner, 1982). The samples were concentrated and the urea removed by ion-exchange chromatography using an Econo-Pac Q cartridge (1.0 ml) (Bio-Rad Laboratories, UK) with an equilibration buffer containing (in mmol l^{-1}) Tris–HCl, 50 (pH 7.2 at 20 °C); DTT, 1; and eluted with a linear concentration gradient of KCl (0 mmol l^{-1} to 300 mmol l^{-1}). Prior to use, all samples were dialysed overnight in (mmol l^{-1}): Tris–HCl, 10; DTT, 1 and 0.1% SDS (w/v) (pH adjusted to 6.75 at 20 °C).

Capillary electrophoresis (CE) was performed with a Biofocus 3000 apparatus (BioRad Laboratories Ltd, UK) using uncoated fused-silica capillary tubes (30 cm×50 mm) (Sigma, Poole, UK) as described by Crockford and Johnston (1995). In order to calculate relative migration times, samples were run against an internal standard (benzoic acid, 0.1 mg ml^{-1}). Standard proteins (molecular masses 45, 36, 20 and 14 kDa) were used to determine the apparent relative molecular mass of the myosin light chains.

Statistical analyses

The results are presented as means ± standard errors (S.E.M.). All data were analysed for normal distribution by use of *N*-scores before applying any statistical tests (Minitab version 7.2, Minitab Inc., USA). The effects of temperature and thermal acclimation on muscle fibre contractile properties and myofibrillar ATPase activities were analysed using two-way analysis of variance (ANOVA) with repeated measures on one factor (temperature). When a significant difference or interaction was observed, further statistical analysis was by *post-hoc* Tukey tests. The ratios of LC3 to LC1 in 5 °C-acclimated and 15 °C-acclimated fish were compared using one- and two-tailed *t*-tests.

Results

Contractile properties of skinned muscle fibres

Skinned fibres isolated from 5 °C-acclimated fish relaxed completely following maximum activations at 0–10 °C (Fig. 1A). At 15 °C and above, fibres from cold-acclimated fish failed to relax completely, showing increasing amounts of Ca^{2+}-independent residual tension (Fig. 1A). Residual force was maintained for long periods (>5 min) (not illustrated). Residual tension was observed only rarely at 15 °C in fibres from 15 °C-acclimated fish and was substantially lower at 20 °C than in fibres from 5 °C-acclimated fish (Fig. 1B). The effects of acute temperature change and thermal acclimation on maximum isometric tension (F_{max}) were analysed using a two-way ANOVA. There was a main effect of acute temperature change on F_{max} ($F_{4,50}=3.34$; $P<0.05$) but the effects of thermal acclimation state ($F_{1,50}=1.88$) and the

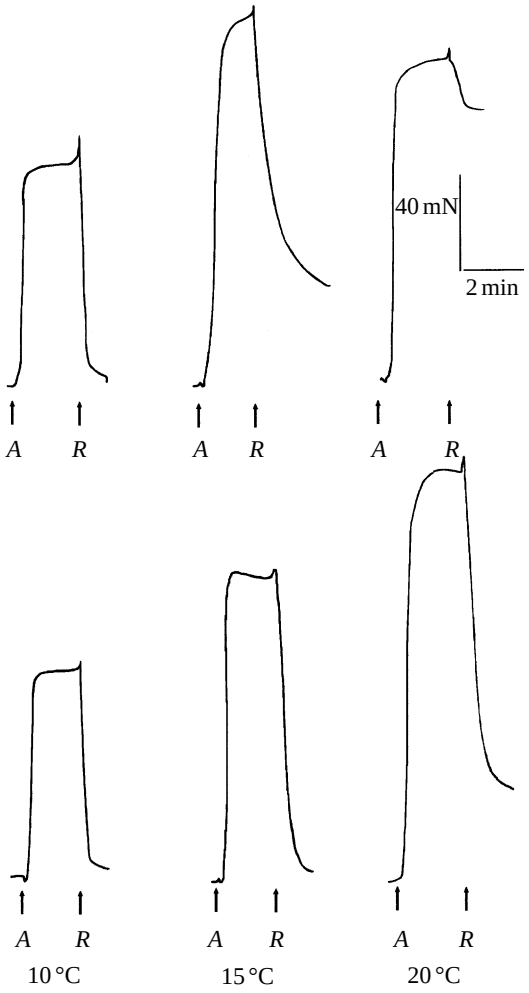


Fig. 1. Force development by skinned fast muscle fibres isolated from short-horned sculpin (*Myoxocephalus scorpius*) acclimated to either 5 °C (A) or 15 °C (B). The arrows indicate transfer from relaxing (*R*) to activating (*A*) solutions, and back to relaxing solution following the attainment of maximum force. Fibres from the 5 °C-acclimated fish showed higher levels of residual tension at 15 and 20 °C, following maximal activation, than fibres from 15 °C-acclimated fish. 10 fibres per temperature from 10 fish.

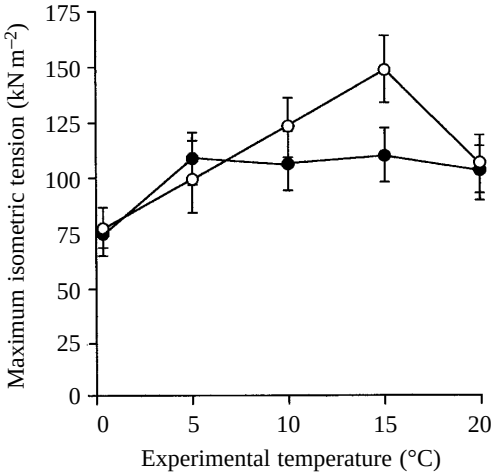


Fig. 2. The effect of temperature on maximum isometric tension (F_{max}) in skinned fibres isolated from the fast myotomal muscle of short-horned sculpin (*Myoxocephalus scorpius*) acclimated to either 5 °C (open circles) or 15 °C (filled circles). Values represent means ± S.E.M.; 10 fibres per temperature from 10 fish.

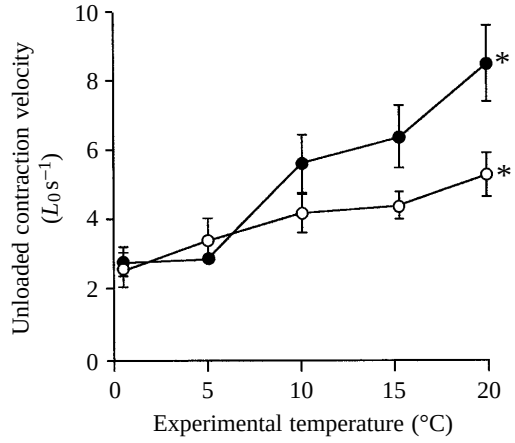


Fig. 3. The effect of temperature on unloaded contraction velocity (V_{slack}) in skinned fibres isolated from the fast myotomal muscle of short-horned sculpin (*Myoxocephalus scorpius*) acclimated to either 5 °C (open circles) or 15 °C (filled circles). Values represent means ± S.E.M.; 10 fibres per temperature from 10 fish. Asterisks mark significantly different values (P<0.05).

interaction between acclimation state and acute temperature change (F4,50=1.12) were not significantly different. *Post-hoc* Tukey tests revealed that, in fibres from 5 °C-acclimated fish, F_{max} was significantly higher at 15 °C (P<0.01) (Fig. 2). However, F_{max} values at 0 and 20 °C were around 75 kN m^{-2} and 110 kN m^{-2}, respectively, in both acclimation groups (Fig. 2).

At 0 °C, the unloaded contraction velocity (V_{slack}) of fast muscle fibres was around 2.8±0.4 fibre lengths s^{-1} (L_0 s^{-1}) in both 5 °C- and 15 °C-acclimated fish. However, the temperature-dependence of V_{max} over the range 0–20 °C was significantly greater in 15 °C-acclimated (Q_{10}=1.76) than in 5 °C-acclimated fish (Q_{10}=1.38) (F4,45=19.21, P<0.01; F1,45=5.62, P<0.05) (Fig. 3). At 20 °C, V_{max} was 39 % higher in fibres from 15 °C-acclimated (8.7±0.9 L_0 s^{-1}) than 5 °C-acclimated fish (5.3±0.6 L_0 s^{-1}) (Fig. 3) (P<0.05). A significant interaction between temperature and thermal acclimation was also found (F4,45=2.84, P<0.05).

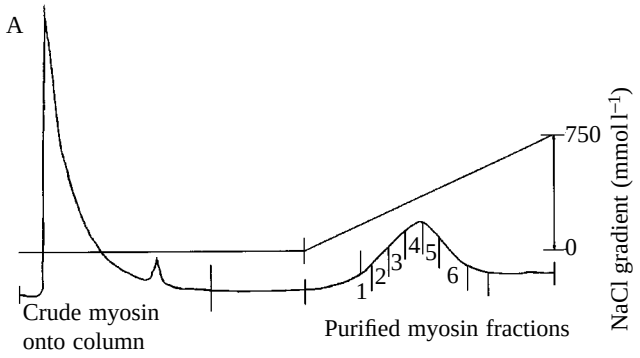
Myofibrillar ATPase activity

Myofibrillar ATPase activity in fast muscle was three times higher at 15 °C than at 5 °C in both acclimation groups (Table 1, P<0.01). There were no significant differences in ATPase activity between acclimation groups at either 5 °C (P<0.15, t=1.68) or 15 °C (P<0.58, t=0.60). Ca^{2+} sensitivity was also similar at both temperatures and across acclimation groups (Table 1).

Table 1. *ATPase activity and Ca^{2+} sensitivity of fast muscle myofibrils from short-horned sculpin acclimated to either 5 °C or 15 °C*

	Experimental temperature (°C)	
	5	15
Myofibrillar ATPase activity (μmol P_i mg^{-1} min^{-1})		
5 °C-acclimated	0.16±0.01	0.48±0.06
15 °C-acclimated	0.13±0.01	0.44±0.06
Ca^{2+} sensitivity (%)		
5 °C-acclimated	92.2±1.7	85.1±2.4
15 °C-acclimated	93.0±2.3	85.4±1.6

Values represent means ± S.E.M. (six fish per acclimation temperature). There were no significant differences in ATPase activity between acclimation groups at either temperature.



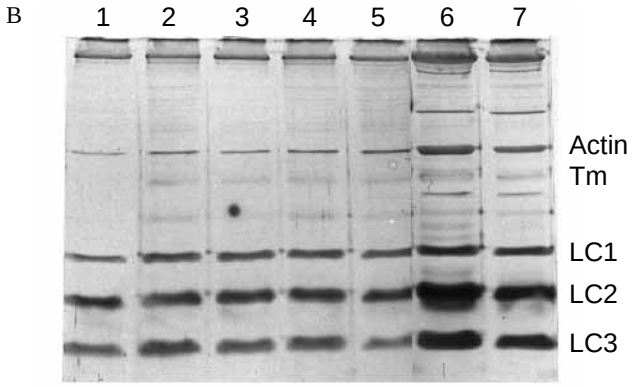


Fig. 4. (A) Purification of myosin from the fast muscle of a 15 °C-acclimated short-horned sculpin by ion-exchange chromatography. The myosin was eluted with a linear gradient of NaCl (see text for details). The numbers refer to the fractions collected and analysed by SDS–PAGE (below). (B) Silver-stained 14% SDS–PAGE gel of the purified myosin (tracks 1–5) and the starting material (tracks 6 and 7).

Myofibrillar protein composition

Purification of myosin by ion-exchange chromatography significantly reduced contaminating levels of actin and tropomyosin (Fig. 4) (compare Fig. 4 with Fig. 5). The

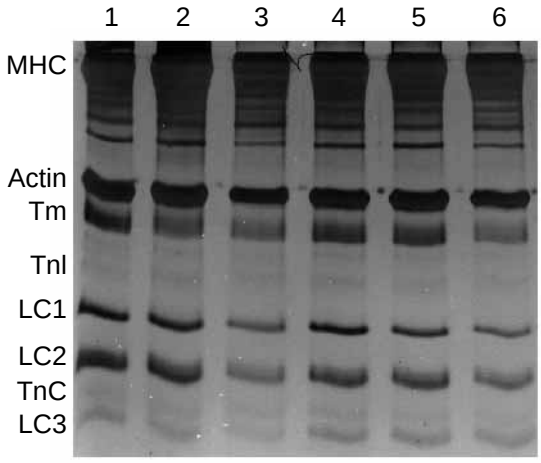


Fig. 5. 14% SDS–PAGE gels of myofibrils isolated from the fast myotomal muscle of short-horned sculpin acclimated to either 5 °C (bands 1–3) or 15 °C (bands 4–6). The gel was stained with Coomassie Brilliant Blue G-250 (see text for details). MHC, myosin heavy chains; Tm, tropomyosin; LC1 and LC3, alkali light chains of myosin; LC2, regulatory myosin light chain; TnI, troponin I; TnC, troponin C.

apparent molecular masses of the major myofibrillar proteins were similar in 15 °C- and 5 °C-acclimated fish; myosin heavy chains (MHC), 220 kDa; actin, 43.4 kDa; tropomyosin (Tm), 33.5 kDa; troponin I (TnI), 26.5 kDa; myosin $LC1_f$, 22.5 kDa; myosin $LC2_f$, 15.5 kDa, troponin C (TnC), 13.3 kDa; myosin $LC3_f$, 12.3 kDa (Fig. 5). (Note that TnT co-migrates with tropomyosin.) There were also no differences in any of the myofibrillar isoforms between acclimation groups as judged by two-dimensional electrophoresis of the acidic and basic proteins (Fig. 6A–D).

Peptide mapping of myosin heavy chains

Characteristic peptide maps of the myosin heavy chains were produced with each protease (Fig. 7A–D). The enzymes used cleave the carboxylic acid side of the following peptide bonds: *Staphylococcus aureus* V8, glutamic acid and aspartic acid; Lys-C, lysine; 1-S chymotrypsin, lysine and arginine; papain cleaves at non-specific sites. Since protease activity is reduced in the presence of SDS, it is possible that not all the sites are cleaved. Good digestion of the myosin heavy chains was obtained with all four proteases. However, no significant differences in the peptide maps of the myosin heavy chains were observed between acclimation groups with any of the protease enzymes used.

Quantification of myosin light chain ratio

SDS–capillary electrophoresis (SDS–CE) provides a sensitive technique for the quantification of myosin light chains (Crockford and Johnston, 1995). No differences were noted in the migration of myosin light chain isoforms isolated from 5 °C- and 15 °C-acclimated fish relative to the benzoic acid standard (Fig. 8A,B). Myosin light chain 2 has been reported to show anomalous migration characteristics on both SDS–PAGE (Huriaux and Focant, 1985) and SDS–CE

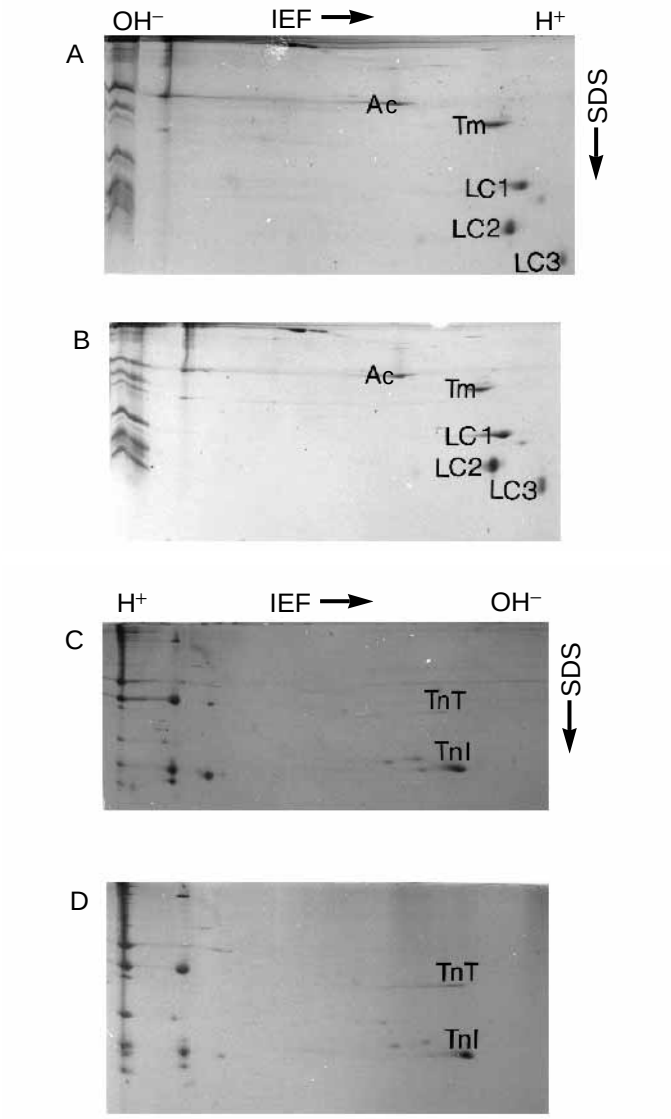


Fig. 6. Two-dimensional PAGE of (A,B) the acidic and (C,D) the basic myofibrillar proteins from 5 °C-acclimated (A,C) and 15 °C-acclimated (B,D) short-horned sculpin. The gel was stained with Coomassie Brilliant Blue G-250 (see text for details). Ac, actin; Tm, tropomyosin; TnT, troponin T; LC1 and LC3, alkali light chains of myosin; LC2, regulatory myosin light chain, TnI, troponin I; IEF, isoelectric focusing.

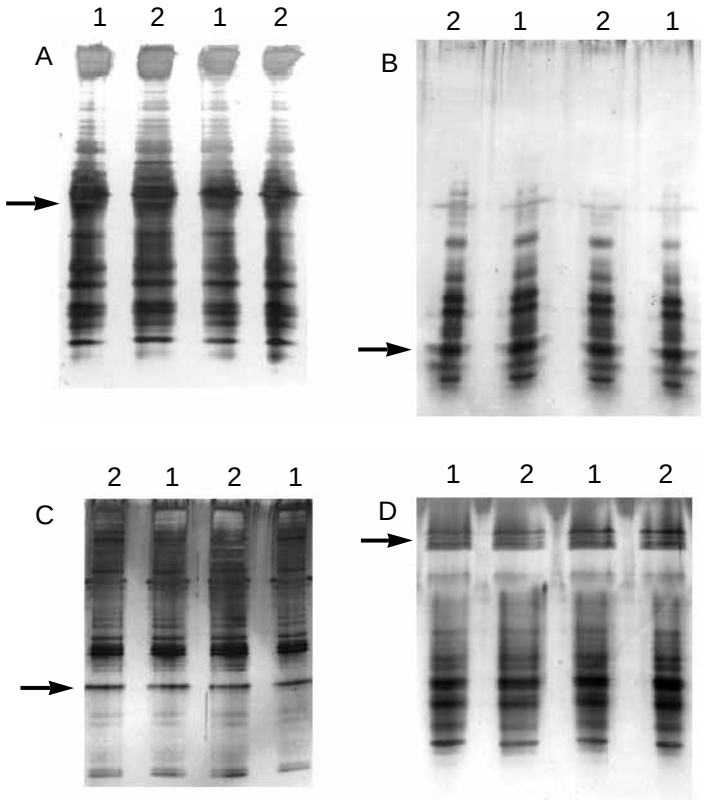


Fig. 7. Peptide maps of purified myosin heavy chains separated on 15% SDS–PAGE gels. The myosins were digested with (A) *Staphylococcus aureus* V8 protease, (B) 1-S chymotrypsin, (C) Lys-C and (D) papain. The arrows indicate bands corresponding to the protease. Myosin heavy chains were isolated from 5 °C-acclimated fish (track 1) and 15 °C-acclimated fish (track 2).

(Crockford and Johnston, 1995). Purified LC2 was used to determine the migration order of the myosin light chains (not illustrated). The apparent molecular masses of the three myosin light chains were ($LC1_f$, 23.2±1.8 kDa; $LC2_f$, 12.3±2.0 kDa; $LC3_f$, 18.3±2.1 kDa) (means ± S.E.M., 12 fish from both acclimation temperatures combined). The ratio of $LC3_f$ to $LC1_f$ contents was found to be 0.73±0.10 in 15 °C-acclimated fish and 1.66±0.27 in 5 °C-acclimated fish (P<0.005) (six fish per acclimation temperature; Fig. 8A,B). Thus, there was approximately 50% more LC3 in myosin from cold- than from warm-acclimated fish: 5 °C fish, $L3_f/(LC3_f+LC1_f)$=0.61; 15 °C fish, $LC3_f/(LC3_f+LC1_f)$=0.40.

Discussion

Isometric tension

There were no significant differences in the relationship between temperature and maximum isometric tension (F_{max}) for skinned muscle fibres isolated from 5 °C- and 15 °C-acclimated short-horned sculpin (Fig. 2). F_{max} was 75 kN m^{-2} at 0 °C and 110 kN m^{-2} at 20 °C in both acclimation groups (Fig. 2). In contrast, marked differences have been reported in the relationship between maximum tetanic tension and temperature for live muscle fibre bundles in the same species (Beddow and Johnston, 1995). Absolute values for maximum tension were around two times higher for live than for skinned fast muscle fibres of the short-horned sculpin; similar findings have been reported for other fish (Bone *et al.* 1986; Curtin and Woledge, 1987). Maximum tetanic tension increased from 125 kN m^{-2} at 5 °C to 282 kN m^{-2} at 15 °C in 15 °C-acclimated fish (Beddow and Johnston, 1995). However, live fibres from 5 °C-acclimated fish generated much lower maximum tensions at 15 °C (78 kN m^{-2}, Beddow and Johnston, 1995). Following depolarisation with a high-K^+ solution (190 mmol l^{-1}), the maximum force in fibres from 5 °C-acclimated fish was more than twofold higher at 15 °C, consistent with some failure in

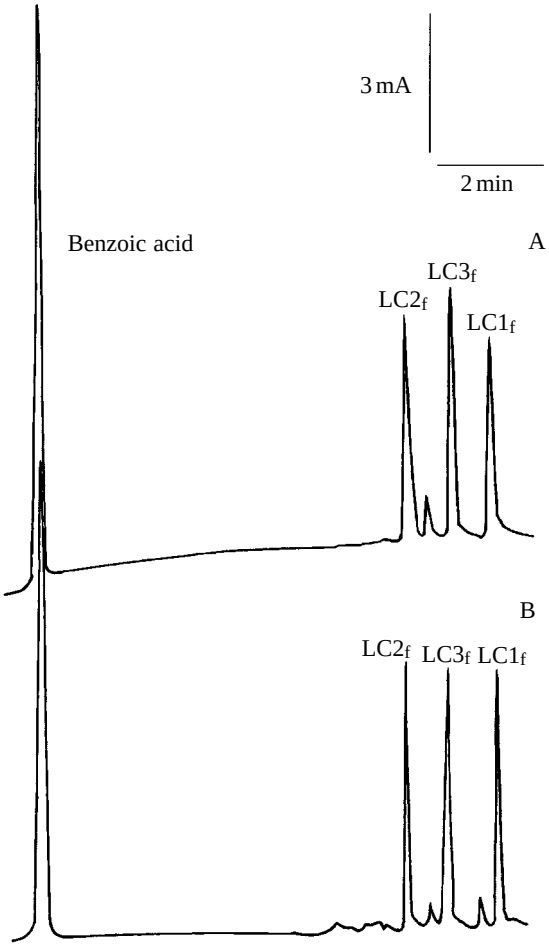


Fig. 8. Separation of myosin light chains from the fast muscle of a 15 °C-acclimated short-horned sculpin by SDS–capillary electrophoresis using an internal benzoic acid standard (see text for details). (A) Purified myosin from 5 °C-acclimated fish; (B) purified myosin from 15 °C-acclimated fish. $LC1_f$ and $LC3_f$, alkali light chains of myosin; $LC2_f$, regulatory myosin light chain.

activation and/or excitation–contraction coupling with tetanic stimulation. Fast muscle fibres in this species become inexcitable on treatment with α-bungarotoxin, which suggests that activation is *via* the release of acetylcholine from the muscle endplates (Johnson *et al.* 1991). Significant presynaptic effects of temperature acclimation on the neuromuscular junction have previously been reported for carp (Harper *et al.* 1989). The present study has shown that the failure of muscle fibres from cold-acclimated short-horned sculpin to generate high tensions at 15 °C does not reflect an intrinsic property of the contractile proteins. In contrast, in the common carp (*Cyprinus carpio*), thermal acclimation affects maximum tension generation of both skinned (Johnston *et al.* 1985) and live (Langfeld *et al.* 1991) fibres. At 7 °C, skinned fast fibres isolated from 7 °C-acclimated carp generated 178 $kN\,m^{-2}$, compared with only 76 $kN\,m^{-2}$ for fibres from 23 °C-acclimated fish. F_{max} was 209 $kN\,m^{-2}$ at 23 °C in warm-acclimated carp, whereas fibres from cold-acclimated fish showed a rapid decline in force following maximal activations at this temperature (Johnston *et al.* 1985). As in the present study, maximally activated skinned fibres from cold-acclimated carp showed significant levels of residual tension at high temperatures (above 20 °C) following transfer to relaxing solution. Thus, in carp, thermal acclimation results in major differences in the force-generating phenotype of the contractile proteins over and above any effects on muscle membranes and/or presynaptic transmission.

Maximum contraction velocity

In the present study, both temperature and thermal history had a significant effect on the maximum unloaded contraction velocity (V_{slack}) of single fast skinned muscle fibres (Fig. 3). V_{slack} increased from about 2.8 $L_0\,s^{-1}$ at 0 °C, to 5.3 and 8.7 $L_0\,s^{-1}$ at 20 °C in 5 °C- and 15 °C-acclimated fish respectively. Comparable findings were reported for V_{slack} and V_{max} (determined from the force–velocity relationship) in live fast muscle fibres from the short-horned sculpin acclimated to either 5 or 15 °C (Beddow and Johnston, 1995). For example, V_{slack} was 4.7 $L_0\,s^{-1}$ at 5 °C in both acclimation groups, and at 15 °C V_{slack} was 5.2 $L_0\,s^{-1}$ in 5 °C-acclimated and 8.2 $L_0\,s^{-1}$ in 15 °C-acclimated fish. Thermal acclimation has also been reported to modify V_{max} in fast muscle fibres of the common carp (*Cyprinus carpio*) (Johnston *et al.* 1985). At 7 °C, V_{max} was twofold higher in single skinned fast fibres from 7 °C- than from 23 °C-acclimated carp (Johnston *et al.* 1985).

Molecular mechanisms underlying the plasticity of muscle contractile properties

Bárány (1967) reported a positive correlation between the maximum shortening velocity of a muscle and its myosin ATPase activity. More recently Bottinelli *et al.* (1994) measured isometric ATPase activity and unloaded shortening velocity in the same single-fibre segments from rat hindlimb muscles. They reported that fibres with the highest levels of ATPase activity also had the greatest shortening speed and contained fast isoforms (type IIb) of myosin heavy chains. Greaser *et al.* (1988) found that, within a group of the fastest contracting fibres from rat plantaris muscle which contained fast myosin heavy chain isoforms, there was a positive correlation between the ratio of $LC3_f$:$LC1_f$ contents and unloaded shortening speed. Direct evidence that myosin light chains modulate V_{max} was obtained by Lowey *et al.* (1993) using an *in vitro* motility assay in which the velocity of actin filaments sliding over myosin-coated glass slides was determined. Removal of the light chain subunits reduced the velocity of actin filaments from 8.8 to 0.8 $mm\,s^{-1}$ at 30 °C, without affecting myosin ATPase activity. Insights into the molecular mechanisms of contraction have been greatly advanced by the determination of the three-dimensional atomic structure of rabbit myosin subfragment 1 by X-ray diffraction (Rayment *et al.* 1993). The actin-binding site and the ATP-binding pocket are located 4 nm apart on the globular head region of the molecule, whilst the myosin light chains are wrapped around an α-helix of 73 amino acid residues towards the carboxy terminus. It has been suggested

the light chains function to provide rigidity to this α-helix, enabling it to act as a 'lever arm'. The working stroke of the lever arm has been calculated to be of the order of 10 nm, which is close to the step size of single myosin molecules as measured in laser trap experiments (Finer *et al.* 1994). Genetically engineered slime moulds, *Dictyostelium* sp., with a 50 % shortened myosin lever arm were found to have 50 % lower actin sliding velocities in an *in vitro* motility assay (Uyeda and Spudich, 1993). Crockford *et al.* (1995) have reported an allelic variation in myosin $LC1_f$ in the fast myotomal muscle fibres of the tropical cichlid *Oreochromis andersonii* which was correlated with a difference in maximum contraction velocity. Fibres from individuals with the smaller myosin light chain 1 had a 33 % faster maximum contraction velocity. Since no other differences in myofibrillar isoforms were detected between $LC1_f$ phenotypes, these results are consistent with light chains functioning to modulate contraction velocity in intact muscle fibres in the absence of any change in myosin heavy chain composition. The results of the present study are consistent with a similar mechanism operating to modulate V_{max} with temperature acclimation in the short-horned sculpin. The ratio of $LC3_f$:$LC1_f$ contents was 1.66 in 5 °C-acclimated fish and 0.73 in 15 °C-acclimated fish, as determined by capillary electrophoresis. Peptide mapping with four different enzymes provided no evidence for any differences in the myosin heavy chain composition between acclimation groups. Changes in myosin light chain expression in the absence of altered myosin heavy chain composition would be expected to alter V_{max} without affecting ATPase activity, as reported in Table 1. In birds and mammals, myosin light chains $LC3_f$ and $LC1_f$ are produced from a single gene by an alternate RNA transcription and splicing mechanism, and differ only in their N-terminal sequences (Periasamy *et al.* 1984; Nabeshima *et al.* 1984). In contrast, in the mullet *Mugil cephalus*, 29 amino acid differences were found between LC1 and LC3, spread throughout the polypeptide chain, suggesting that they may be the products of different genes.

The molecular mechanism underlying changes in V_{max} with temperature acclimation in the short-horned sculpin is different from that reported for common carp. In carp, temperature acclimation results in changes in the expression of both myosin heavy chains (Gerlach *et al.* 1990; Hwang *et al.* 1991) and light chains (Crockford and Johnston, 1990). The finding of altered myosin heavy chain composition with temperature acclimation in cyprinids explains the observed changes in myofibrillar ATPase activity (Johnston *et al.* 1975; Heap *et al.* 1986; Johnson and Bennett, 1995) and in force generation of skinned muscle fibres (Johnston *et al.* 1985). Crockford and Johnston (1990) found evidence for a unique myosin light chain 3 isoform ($LC3_c$) in cold-acclimated carp. In addition, the total myosin light chain 3 content in fast muscle was significantly greater in 8 °C- than in 20 °C-acclimated fish (Crockford and Johnston, 1990).

Food intake and growth rate generally increase with acclimation temperature to an optimum and then decline (Jobling, 1993). Enion *et al.* (1995) found that fast muscle in warm-acclimated carp expressed a myosin heavy chain gene thought to be associated with hyperplasic growth in adult fish. Thus, there may be confounding effects of growth and temperature-acclimation on myosin gene expression in fish. Nevertheless, sequencing and peptide mapping studies of light meromyosin (Watabe *et al.* 1995) and subfragment-1 (Hwang *et al.* 1991) have revealed significant differences in the major isoforms present in the muscle of cold- and warm-acclimated common carp.

The present study has shown that the molecular mechanisms underlying the plasticity of contractile properties with temperature acclimation differ in the only two species studied in detail. The Cottidae are thought to have originated in the North Pacific and undergone a major radiation to fill a variety of niches in fresh water and sea water in both North America and Europe. The group includes highly stenothermal arctic species and eurythermal species such as the freshwater bullead (*Cottus gobio*), which can experience a seasonal temperature range from 3 to 30 °C. In order to test different hypotheses about the evolution of phenotype plasticity, it would be highly informative to obtain an independent estimate of evolutionary relationships within the family Cottidae and to map onto it information about temperature-acclimation responses at the whole-animal, physiological and molecular levels.

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References

ALTRINGHAM, J. D. AND JOHNSTON, I. A. (1982). The pCa–tension and force–velocity characteristics of skinned fibres isolated from fish fast and slow muscles. *J. Physiol., Lond.* **333**, 421–449.

ALTRINGHAM, J. D. AND JOHNSTON, I. A. (1990). Scaling effects on muscle function: power output of isolated fish muscle fibres performing oscillatory work. *J. exp. Biol.* **151**, 453–467.

BÁRÁNY, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *J. gen. Physiol.* **50**, 197–218.

BEDDOW, T. A. AND JOHNSTON, I. A. (1995). Plasticity of muscle contractile properties following temperature acclimation in the marine fish *Myoxocephalus scorpius*. *J. exp. Biol.* **198**, 193–201.

BEDDOW, T. A., VAN LEEUWEN, J. L. AND JOHNSTON, I. A. (1995). Swimming kinematics of fast-starts are altered by temperature acclimation in the marine fish *Myoxocephalus scorpius*. *J. exp. Biol.* **198**, 203–208.

BONE, Q., JOHNSTON, I. A., PULSFORD, A. AND RYAN, K. P. (1986). Contractile properties and ultrastructure of three types of muscle fibre in the dogfish myotome. *J. Muscle Res. cell. Motil.* **7**, 47–56.

BOTTINELLI, R., BETTO, R., SCHIAFFINO, S. AND REGIANNI, C. (1994). Unloaded shortening velocity and myosin heavy chain and alkali light chain isoform composition in rat skeletal muscle fibres. *J. Physiol., Lond.* **478**, 341–349.

CLEVELAND, D. W., FISCHER, S. G., KIRSCHNER, N. W. AND LAEMMLI, U. K. (1977). Peptide mapping by limited proteolysis in sodium

dodecyl sulphate and analysis by electrophoresis. *J. biol. Chem.* **2525**, 1102–1106.

CROCKFORD, T. AND JOHNSTON, I. A. (1990). Temperature acclimation and the expression of contractile protein isoforms in the skeletal muscles of the common carp (*Cyprinus carpio* L.). *J. comp. Physiol.* B **160**, 23–30.

CROCKFORD, T. AND JOHNSTON, I. A. (1995). Isolation of unstable myosins and the analysis of light chains by capillary electrophoresis. *Analyt. Biochem.* **231**, 20–26.

CROCKFORD, T., JOHNSTON, I. A. AND MCANDREW, B. J. (1995). Functionally significant allelic variation in myosin light chain composition in a tropical cichlid. *J. exp. Biol.* **198**, 2501–2508.

CURTIN, N. A. AND WOLEDGE, R. C. (1987). Force–velocity relation of living fibres from dogfish white muscle. *J. Physiol., Lond.* **387**, 46P.

EDMAN, K. A. P. (1979). Unloaded contraction velocity of single skinned rat muscle fibres. *J. Physiol., Lond.* **291**, 143–155.

EGGINTON, S. AND SIDELL, B. D. (1989). Thermal acclimation induces adaptive changes in subcellular structure of fish skeletal muscle. *Am. J. Physiol.* **256**, R1–R9.

ENION, S., GAUVRY, L., BUTTERWORTH, P. AND GOLDSPINK, G. (1995). Small-diameter white myotomal muscle fibres associated with growth hyperplasia in the carp (*Cyprinus carpio*) express a distinct myosin heavy chain gene. *J. exp. Biol.* **198**, 1603–1611.

FINER, J. T., SIMMONS, R. M. AND SPUDICH, J. A. (1994). Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature* **368**, 113–119.

FLEMING, J. R., CROCKFORD, T., ALTRINGHAM, J. R. AND JOHNSTON, I. A. (1990). Effects of temperature acclimation on muscle relaxation in the carp: A mechanical, biochemical and ultrastructural study. *J. exp. Zool.* **255**, 286–295.

FRY, F. E. J. AND HART, J. S. (1948). Cruising speed of goldfish in relation to water temperature. *J. Fish. Res. Bd Can.* **7**, 169–175.

GERLACH, G.-F., TURAY, L., MALIK, K. T. A., LIDA, J., SCUTT, A. AND GOLDSPINK, G. (1990). Mechanisms of temperature acclimation in the carp: a molecular biology approach. *Am. J. Physiol.* **259**, R237–R244.

GREASER, M. L., MOSS, R. L. AND REISER, L. (1988). Variations in contractile properties of rabbit single muscle fibres in relation to troponin T isoforms and myosin light chains. *J. Physiol., Lond.* **406**, 85–98.

HARPER, A. A., SHELTON, J. R. AND WATT, P. W. (1989). The temperature dependence of the time course of growth and decay of miniature end-plate currents in carp extraocular muscle following thermal acclimation. *J. exp. Biol.* **147**, 237–248.

HEAP, S. P., WATT, P. W. AND GOLDSPINK, G. (1986). Myofibrillar ATPase activity in the carp (*Cyprinus carpio*): interactions between starvation and environmental temperature. *J. exp. Biol.* **123**, 373–382.

HURIAUX, F. AND FOCANT, B. (1985). Electrophoretic and immunological study of myosin light chains from fresh water teleost fishes. *Comp. Biochem. Physiol.* **82**B, 737–743.

HWANG, G. C., OCHIAI, Y., WATABE, S. AND HASHIMOTO, K. (1991). Changes in the carp myosin subfragment-1 induced by temperature acclimation. *J. comp Physiol* B **161**, 141–146.

ITZHAKI, R. F. AND GILL, D. M. (1964). A micro-buiret method for estimating proteins. *Analyt. Biochem.* **9**, 401–410.

JOBLING, M. (1993). Bioenergetics: feed intake and energy partioning. In *Fish Physiology* (ed. J. C. Rankin and F. B. Jensen), pp. 1–44. London: Chapman & Hall.

JOHNSON, T. P., ALTRINGHAM, J. D. AND JOHNSTON, I. A. (1991). The effects of Ca^{2+} and neuromuscular blockers on the activation of fish muscle fibres. *J. Fish Biol.* **38**, 789–790.

JOHNSON, T. P. AND BENNETT, A. F. (1995). The thermal acclimation of burst escape performance in fish: an integrated study of molecular and cellular physiology and organismal performance. *J. exp. Biol.* **198**, 2165–2175.

JOHNSTON, I. A., DAVISON, W. AND GOLDSPINK, G. (1975). Adaptations in Mg^{2+}-activated myofibrillar ATPase activity induced by temperature acclimation. *FEBS Lett.* **50**, 293–295.

JOHNSTON, I. A., FLEMING, J. D. AND CROCKFORD, T. (1990). Thermal acclimation and muscle contractile properties in cyprinid fish. *Am. J. Physiol.* **259**, R231–R236.

JOHNSTON, I. A. AND GLEESON, T. T. (1987). Effects of temperature on contractile properties of skinned muscle fibres from three toad species. *Am. J. Physiol.* **252**, R371–R375.

JOHNSTON, I. A. AND LUCKING, M. (1978). Temperature induced variation in the distribution of different types of muscle fibres in the goldfish (*Carassius auratus*). *J. comp. Physiol.* **124**, 111–116.

JOHNSTON, I. A. AND MAITLAND, B. (1980). Temperature acclimation in crucian carp: a morphometric study of muscle fibre ultrastructure. *J. Fish Biol.* **17**, 113–125.

JOHNSTON, I. A., SIDELL, B. D. AND DRIEDZIC, W. R. (1985). Force–velocity characteristics and metabolism of carp muscle fibres following temperature acclimation. *J. exp. Biol.* **119**, 239–249.

JOHNSTON, I. A., VAN LEEUWEN, J. L., DAVIES, M. L. F. AND BEDDOW, T. (1995). How fish power predation fast-starts. *J. exp. Biol.* **198**, 1851–1861.

JOHNSTON, I. A. AND WALESBY, N. J. (1977). Molecular mechanisms of temperature compensation in fish myofibrillar adenosine triphosphatases. *J. comp. Physiol.* **119**, 195–206.

LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

LANGFELD, K. S., CROCKFORD, T. AND JOHNSTON, I. A. (1991). Temperature acclimation in the common carp: force–velocity characteristics and myosin subunit composition of slow muscle fibres. *J. exp. Biol.* **155**, 291–304.

LEMMONS, D. E. AND CRAWSHAW, L. I. (1985). Behavioural and metabolic adjustments to low temperatures in the largemouth bass (*Micropterus salmoides*). *Physiol. Zool.* **58**, 175–180.

LOWEY, S., WALLER, G. AND TRYBUS, K. M. (1993). Skeletal muscle myosin light chains are essential for physiological speeds of shortening. *Nature* **365**, 454–456.

NABESHIMA, Y., FUJI-KURIYAMA, Y., MURAMATSU, M. AND OGATA, K. (1984). Alternate transcription and two modes of splicing result in two myosin light chains from a single gene. *Nature* **308**, 333–338.

O'FARRELL, P. H. (1975). High resolution two-dimensional electrophoresis of proteins. *J. biol. Chem.* **250**, 4007–4021.

PERIASAMY, M., STREHLER, E. E., GARFINKEL, L. I., GUBITS, R. M., RUIS-OPASO, N. AND NADAL-GINARD, B. (1984). Fast skeletal muscle myosin light chains 1 and 3 are produced from a single gene by a combined process of differential RNA transcription and splicing. *J. biol. Chem.* **259**, 13595–13604.

RAYMENT, I., RYPNIEWSKI, W. R., SCHMIDT-BASE, K., SMITH, R., TOMCHICK, D. R., BENNING, M. M., WINKLEMANN, D. A., WESENBERG, G. AND HOLDEN, H. (1993). Three-dimensional structure of myosin subfragment-1: a molecular motor. *Science* **261**, 50–58.

ROCKSTEIN, M. AND HERRON, P. W. (1955). Colorimetric determination of inorganic phosphate in microgram quantities. *Analyt. Chem.* **23**, 1500–1501.

SIDELL, B. D. (1980). Response of goldfish (*Carassius auratus* L.) to acclimation temperature: alterations in biochemistry and proportions of different fibre types. *Physiol. Zool*. **53**, 98–107.

SIDELL, B. D. AND HAZEL, J. R. (1987). Temperature affects the diffusion of small molecules through cytosol of fish muscle. *J. exp. Biol*. **129**, 191–203.

SISSON, J. E. AND SIDELL, B. D. (1987). Effect of thermal acclimation on muscle fiber recruitment of swimming striped bass (*Morone saxatilis*). *Physiol. Zool*. **60**, 310–320.

UYEDA, T. Q. P. AND SPUDICH, J. A. (1993). A functional recombinant myosin-II lacking a regulatory light-chain binding-site. *Science* **262**, 1867–1870.

WAGNER, P. D. (1982). Preparation and fractionation of myosin light chains and exchange of essential light chains. In *Methods in Enzymology*, vol. 85 (ed. S. P. Colowick and N. O. Kaplan), pp. 72–73. London: Academic Press.

WATABE, S., IMAI, J., NAKAYA, M., HIRAYAMA, Y., OKAMOTO, Y., MASAKI, H., UOZOMI, T., HIRONO, I. AND AOKI, T. (1995). Temperature acclimation induces light meromyosin isoforms with different primary structures in carp fast skeletal muscle. *Biochem. biophys. Res. Commun*. **208**, 118–125.